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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Antipsychotic drugs (APDs) are widely used psychotropic medications, though they have significant metabolic side effects. While the mechanisms for these metabolic disturbances are poorly understood, the single known unifying property of all APDs is their blockade of the dopamine D2 (D2R) and D3 (D3R) receptors. We therefore hypothesize that D2R and/or D3R mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas, areas critical for metabolic regulation. In Year 3 of this award, we have completed the design of a novel inducible D3R-flox mouse in order to selectively knock out expression of D3R in the hypothalamus and pancreatic beta cells. The resulting transgenic mice are being tested to confirm the successful production of the strain. In parallel, we have completed construction of novel inducible transgenic hypothalamic- and pancreatic beta cell-specific D3R knockout (KO) mice. Additionally, using pancreatic islets isolated from beta cell-selective D2R KO mice and whole body D3R KO mice we found diminished inhibition of stimulated insulin secretion in the central nervous system and the periphery relative to littermate controls, suggesting a role for both receptors in mediating insulin secretion. We did not identify major metabolic deficits in central neuronal Nkx 2.1 D3R knockouts relative to thier respective controls.

15. SUBJECT TERMS
NONE LISTED

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1. INTRODUCTION

Antipsychotic drugs (APDs) are widely used psychotropic medications for numerous psychiatric illnesses including schizophrenia, posttraumatic stress disorder and depression. However, these medications also have significant metabolic side effects characterized by substantial weight gain, glucose intolerance, insulin resistance, hypertension and dyslipidemia as well as increased risks for type 2 diabetes and cardiovascular disease. Indeed, the prevalence of these APD-induced metabolic side effects in Veterans is more than twice that of the general population. However, the mechanisms for these metabolic disturbances are not well understood. Significantly, all APDs cause these side effects to differing degrees and ultimately result in life-shortening morbidity. A potentially important clue is that the single known unifying property of all APDs is their blockade of the dopamine D_2 (D2R) and D_3 (D3R) receptors, suggesting a role for these receptors in APD metabolic side effects. Consistent with this, D2R and D3R are expressed both centrally in the hypothalamus in regions mediating appetite and feeding behavior as well as peripherally in insulin-releasing pancreatic beta cells, key regulators of metabolism. We previously showed that activation of pancreatic beta cell D2R and D3R inhibited glucose-stimulated insulin secretion (GSIS) and that APD-induced receptor inhibition disrupted this regulatory mechanism. Thus, our central hypothesis is that D2R and/or D3R are critical regulators of metabolism and mediate the metabolic side effects of APDs both centrally in the hypothalamus and peripherally in pancreas. However, the relative contributions of peripheral and central D2R and D3R to APD-induced metabolic dysregulation are unknown. To disentangle these mechanisms, in partnership with PI Dr. Zachary Freyberg, we will aim to do the following: (1) to identify contributions of hypothalamic D2R and D3R action in APD-induced weight gain and metabolic dysregulation *in vivo*; (2) to identify the relationship of peripheral D2R and D3R to APD-induced weight gain and metabolic dysfunction *in vivo*; and (3) to identify APD-mediated effects on insulin and DA release in pancreatic beta cells using real-time imaging. Key to these aims is the generation of tissue-specific D2R and D3R knockout (KO) mice targeting either hypothalamus or pancreatic beta cells. Moreover, in focusing on the peripheral contributions of pancreatic D2R and D3R, we have also developed new and highly sensitive optical and biochemical assays to study D2R- and D3R-mediated effects on insulin and DA release in real-time. We have applied these new assays to an experimentally tractable model using the well-characterized rat beta cell-derived INS-1E cell line for our *in vitro* studies, in addition to our work in the D2R and D3R KO pancreatic islets. In the short term, our work will elucidate the anatomical and functional mechanisms of APD-induced metabolic side effects. In the longer term, we will use our findings to develop better-targeted APDs that can selectively reverse these drugs' metabolic side effects while preserving their clinical efficacy.

2. KEYWORDS

Keywords relevant to the work proposed here include:

1. Antipsychotic drug (APD)
2. Dopamine (DA)
3. Dopamine D_2 Receptor (D2R)
4. Dopamine D_3 Receptor (D3R)
5. Insulin
6. Glucose-stimulated insulin secretion (GSIS)
7. Diabetes
8. Metabolism

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**
  The major goals of the project as stated in the approved SOW are as follows:
  A. Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment
  B. Metabolic characterization of pancreatic beta cell-specific D2R and D3R knockout mice in the presence or absence of APD treatment
  C. Treatment with domperidone to determine whether peripheral D2R/D3R blockade alone can produce relevant metabolic disease
  D. Determine the precise contributions of D2R and D3R to glucose-stimulated insulin and dopamine release using pancreatic islets from pancreatic beta cell-selective D2R and D3R knockout mice as well as wildtype controls
  E. Determine effects of APDs on kinetics of real-time glucose-stimulated insulin and dopamine release in wildtype and beta cell-specific D2R or D3R knockout mouse pancreatic islets

- **What was accomplished under these goals?**
  - **Metabolic characterization of hypothalamus-specific D2R and D3R knockout mice in the presence or absence of APD treatment**

We have also established and are now successfully breeding hypothalamus-specific D3R knockout mice (Nkx2.1-cre hemizygous, D3R-flox homozygous mice). These animals are currently being maintained on the high fat-high carbohydrate diet that we have used to promote the development of glucose intolerance. Two of the cohorts of male and we have tried failed to develop high levels of glucose intolerance, yet have become more obese. Basal glucose and insulin levels in these animals are elevated, and we have placed the animals in metabolic cages to identify and characterize the role of forebrain-specific D3R in the development of glucose intolerance, food intake, obesity and energy expenditure. We have not yet found significant differences between these hypothalamic D3 knockout animals and their respective genetic background strain controls. We hypothesize that this may in part be due to the relative lack of specificity of the D3 knockdown, which includes non hypothlamic neurons. We have accordingly pursued more hypothalamic specific Cre- mouse lines unique to hypothalamic neuronal populations implicated in the control of blood glucose and energy balance, such as agouti-related protein (AGRP) and proopiomelanocortin (POMC-Cre) mice to develop D2 and D3R flox crosses and thereby target GARP and POMC loss of D2 and D3R, respectively. We have acquired the Agrp-Cre line and are currently breeding these animals. Based on our recently completed and published work in Mip1cre-Drd3-flox beta cell-selective KO animals, we do not anticipate any difficulties in performing these metabolic characterizations.

We also investigated D3R's role in maintaining insulin homeostasis in vivo using our β-cell-selective D3R KO mice. We measured the effects of D3R KO on changes in serum insulin levels in response to a meal challenge. Although there were no significant differences in fasting serum insulin levels between D3R KO and the WT control mice preceding the meal challenge ($P > 0.05$), or on serum insulin levels in D3R KO mice following a meal challenge ($P > 0.05$). We explored the possibilities

that these insulin increases were in response to concomitant elevations in blood glucose and/or increased insulin resistance in the D3R KO mice relative to the WT control animals. Comparisons of fasting or postprandial glucose levels between the two genotypes did not reveal any significant differences ($P > 0.05$). Furthermore, we found no significant differences in insulin sensitivity either at basal or post-glucose infusion timepoints between D3R KO and WT mice as measured by intraperitoneal glucose tolerance testing (ipGTT) ($P > 0.05$) or via calculation of the HOMA-IR ($P > 0.05$; WT: 5.2 ± 1.8, D3R KO: 9.2 ± 1.4). These data suggest that the constituents of the food pellets provided in the oral challenge were not sufficient to generate D3R precursors that are absent when glucose is administered i.p. Importantly, our results also suggest that the differences in postprandial insulin levels may therefore be a consequence of direct changes in insulin secretion rather than in response to insulin resistance or elevated blood glucose levels in the D3R KO mice. Overall, these data suggest that D3R modulation of insulin release is not especially sensitive to acute food intake. Moreover, blunted D3R signaling in β-cells is unlikely to mediate GSIS inhibition or postprandial hyperinsulinemia.

- **Metabolic characterization of pancreatic beta cell-specific D3R knockout mice in the presence or absence of APD treatment**

We are in the process of characterizing the quantity and duration of tamoxifen necessary to induce successful deletion of D3R in our inducible pancreatic beta cell-specific D3R knockout mice. Our current studies have not yet produced reliable significant knockdown, using an intraperitoneal route of administration, 100 mg/kg, every other day (3 times a week) for 1 week, where the tamoxifen is dissolved in prepared 90% corn oil and 10% ethanol mix. We are evaluating alternative vehicles such as dimethyl sulfoxide as the tamoxifen emulsion seems to precipitate and is not well distributed once injected. Once we have confirmed deletion of D3R in pancreatic islets isolated from beta cells, we will begin characterizing the metabolic status of these animals from week 3 of life onwards following completion of weaning. Specifically, we will conduct weekly measurement of weights and food consumption in beta cell-specific D3R (and wildtype littermate controls) treated with either with first-generation APD haloperidol or second-generation APD olanzapine (via i.p. administration). We will also measure serum fasting glucose and insulin levels in hypothalamus-specific D3R knockout mice and wildtype littermate control mice in the presence or absence of APD treatment; serum will be collected at weeks 13 and 26 of APD treatment. Once this is complete, we will begin a similar process for pancreatic beta cell-specific D3R knockout mice.

- **What opportunities for training and professional development has the project provided?**
  Nothing to Report.

- **How were the results disseminated to communities of interest?**

Work resulting from this award have recently been published in Molecular Psychiatry documenting for the first time the direct involvement of both D2 and D3 dopamine receptors in the control of insulin secretory evens in the beta cell.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**
  The identification of our novel findings and this signaling pathway has spurred interest by other major diabetes and obesity research teams in evaluating both the peripheral and central effects of D2 and D3 stimulation in diabetes. For example, recent work from investigators at the University of Michigan Diabetes Center has revealed that bromocriptine improves glucose tolerance independent of circadian timing, prolactin, or the melanocortin-4 receptor (Am J Physiol Endocrinol Metab. 2020 Jan 1;318(1):E62-E71. doi: 10.1152/ajpendo.00325.2019), and Dr. Schwartz has communicated with this laboratory to begin to develop collaborative efforts to identify the extent to which that central D2/D3 R and peripheral beta cell D2/D3R may be implicated in these bromocriptine effects.

  What was the impact on other disciplines?
  In the longer term, the knowledge resulting from our work may directly lead to development of better APDs free of metabolic side effects. This could significantly reduce serious morbidity and mortality from medication-associated type II diabetes and cardiovascular disease. Moreover, better understanding the mechanisms by which dopamine and dopamine receptors mediate insulin release may also significantly contribute to our fundamental understanding of obesity and lead to novel treatments. Since APD-induced metabolic disturbances also increase risks of developing type II diabetes and Alzheimer's disease, further elucidating the mechanisms of APD-induced weight gain may also lead to fundamental insights into the mechanisms for development of these disorders.

- **What was the impact on technology transfer?**
  Nothing to Report.

- **What was the impact on society beyond science and technology?**
  Nothing to Report.

5. CHANGES/PROBLEMS

Nothing to Report.

6. PRODUCTS

- **Publications, conference papers, and presentations**

Journal publications

Farino ZJ, et al. New roles for dopamine D2 and D3 receptors in pancreatic beta cell insulin secretion. Mol Psychiatry. 2019 Jan 9. doi: 10.1038/s41380-018-0344-6.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

• Name:	Gary Schwartz
• Project Role:	Principal Investigator
• Researcher Identifier (*e.g.* ORCID ID):	ORCID ID: 0000-0003-0446-5553
• Nearest person month worked:	3
• Contribution to Project:	Dr. Schwartz has designed performed and analyzed all experimental data in the areas of metabolic and behavioral assessments of dopamine action at pancreatic and central neural sites.
• Funding Support:	National Institutes of Health/ R01

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report.

- **What other organizations were involved as partners?**

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

- **Collaborative Awards**

We have worked with the Partnering PI of this award, Dr. Zachary Freyberg.

9. APPENDICES

None.